THE INHIBITION BY ETHIONINE OF THE ENHANCEMENT OF ASCORBIC ACID EXCRETION BY BARBITAL AND BY 3-METHYLCHOLANTHRENE IN THE RAT

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The ability of chemicals to stimulate the excretion of ascorbic acid acid in the rat has been shown to involve an enhancement of the conversion of hexoses into ascorbic acid and into its precursors D-glucuronate and L-gulonate (Evans, et al., 1960). The characteristics of the phenomenon, such as the 2 to 3 day lag in the effect of 3-methylcholanthrene (Conney and Burns, 1959), as well as the fact that many of the compounds increase the activity of several microsomal enzyme systems not directly involved in carbohydrate metabolism (Conney, et al., 1959), suggest that the enhanced ascorbic acid formation results from higher enzyme levels induced by the chemicals. To test this hypothesis, we have investigated the effect of ethionine on the action of two substances of unrelated chemical structure and pharmacological action, namely, barbital and 3-methylcholanthrene. The influence of barbital on ascorbic acid biosynthesis has been extensively studied, while 3-methylcholanthrene has recently been found to be the most active of all substances tested in this system (Burns, et al., 1960).

Male Wistar rats weighing between 280 and 380 g. were allowed to drink evaporated milk-water (1:1) ad libitum for the entire course of the experiment. They were kept in individual metabolism cages and the urine

was collected into 5 ml. of 10 per cent oxalic acid solution. Urinary ascorbic acid was determined daily by the 2,6-dichlorophenolindophenol procedure and frequently by the 2,4-dinitrophenylhydrazine method, both as described by Roe (1954). The results from the two methods were consistently in agreement. The incorporation of DL-ethionine into the diet of ethionine-treated animals was done to saturate the animals with the antagonist. This was believed especially important in experiments with methylcholanthrene, because the hydrocarbon is such a potent stimulator of ascorbic acid excretion and also because its effect is a delayed one.

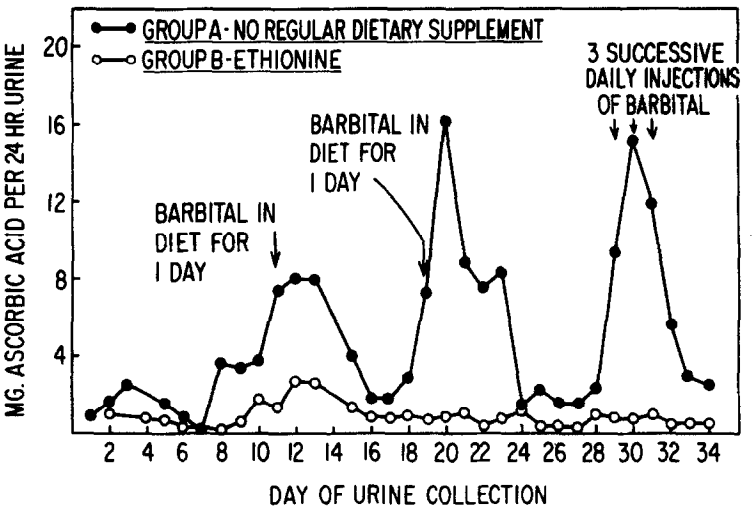

Fig. 1. Influence of DL-ethionine on the barbital enhancement of ascorbic acid excretion by the rat. Average values (2,6-dichlorophenolindophenol method) for the three animals in each group. Group A received only the milk diet, whereas Group B received approximately 75 mg. of ethionine per animal per day in the milk throughout the experiment. Both groups received barbital as indicated. In the first 2 challenges, the drug was included in the diet during days 11 and 19, respectively. In the third test, the drug was injected at the beginning of days 29, 30, and 31. (See text for dosage.)

The responses of control and ethionine-treated rats to 3 challenges by barbital are shown in Fig. 1. In the first two tests, in which the barbital was added to the diet for one day, the presence of both barbital and

ethionine in the milk decreased the food intake sufficiently so as to make the dosage of barbital unequal between the control and ethionine-treated groups[1]. In the third challenge, the drug was injected intraperitoneally (20 mg. of sodium barbital in 1 ml. of water to each animal daily) on 3 successive days to insure equal dosage. It is obvious that ethionine prevents the enhancement of ascorbic acid excretion in all three tests.

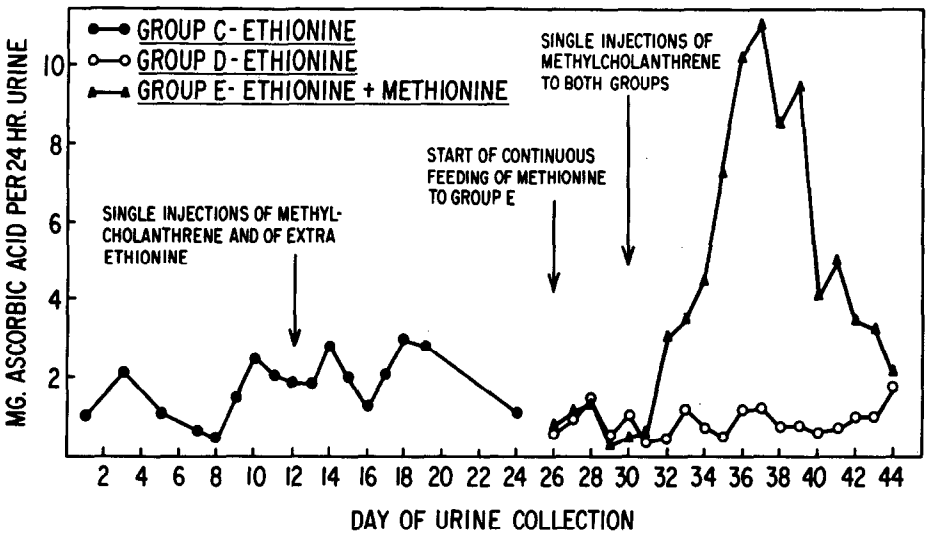

Fig. 2. Influence of DL-ethionine and of DL-ethionine plus DL-methionine on the 3-methylcholanthrene enhancement of ascorbic acid excretion by the rat. Average values (2,6-dichlorophenolindophenol method) for each group. All animals ingested approximately 75 mg. of ethionine per day in milk diet during the entire course of the experiment. At the beginning of days 12, 26, and 30, additional treatment was given as indicated on the graph. The 10 mg. of 3-methylcholanthrene (Mann) was injected intraperitoneally in 0.5 ml. of corn oil, the extra 50 mg. of ethionine on day 12 was injected in 1.0 ml. of 0.9% saline, and the feeding of methionine to Group E involved addition of this amino acid to the milk to give the same concentration as ethionine. Group C consisted of 6 animals. Three of these animals became Group D, 2 became Group E, and 1 was omitted from the second stage of the experiment.

It is clear from the data in Fig. 2 that ethionine also prevents the response to 3-methylcholanthrene. All animals received ethionine throughout the experiment. The ethionine-treated groups C and D failed to respond

[1] Average barbital intake in these tests: control, 111 and 100 mg. per animal; ethionine-treated, 70 and 30 mg. per animal.

to the hydrocarbon, whereas group E, which ingested DL-methionine as well as DL-ethionine, showed greatly increased urinary ascorbic acid levels. In control animals (not shown in graph), supplementary methionine had no effect on ascorbic acid excretion when no drug was administered. The response of group E to methylcholanthrene was not as great or as prolonged as that of animals on milk alone, but higher dosage of methionine probably would have nullified completely the ethionine block.

In a subsequent experiment, a single injection of 50 mg. of ethionine 30 minutes before administration of methylcholanthrene diminished somewhat the extent and duration of the effect of a single treatment with the hydrocarbon.

There are several explanations for the known effects of ethionine on metabolism. One is that it substitutes for methionine in protein synthesis, presumably yielding an inactive product (Shive and Skinner, 1958). In rats, ethionine prevents induction of a hydroxylating enzyme by 3,4-benzpyrene (Conney, et al., 1957) and diminishes the induced formation of a liver protein which binds 3-methyl-4-dimethylaminoazobenzene (Gelboin, et al., 1958). In addition, the induced formation of azo dye demethylase by 3-methylcholanthrene and by phenobarbital is prevented by pretreatment with ethionine (Conney, et al., 1956; Conney and Burns, 1959). In all three systems, the simultaneous administration of methionine nullifies the effect of the antagonist. The findings of the present study are consistent with the similar interpretation that the enhanced excretion of ascorbic acid induced by drugs and other chemicals involves protein biosynthesis, presumably of enzymes concerned with the formation of the vitamin. Further investigations are necessary to disclose the specific transformations concerned.

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